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Short communication

Pharmacology of quinpirole-stimulated [^{35}S]GTPγS binding: discrepancy with receptor binding profile

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Abstract

Functional consequences of receptor stimulation by quinpirole, a dopamine D_2-like receptor agonist, were assessed using agonist-stimulated [^{35}S]GTPγS binding in rat striatal membranes. Dopamine receptor antagonists inhibited quinpirole-stimulated [^{35}S]GTPγS binding with the following rank order of potency: spiperone > haloperidol > clozapine > SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine), consistent with a dopamine D_2-like profile. In contrast, the monoamine oxidase inhibitors Ro 41-1049 (*N*-(2-aminoethyl)-5-(3-fluorophenyl)-4-thiazolecarboxemide), and (+)- and (−)-deprenyl, which inhibit [^{3}H]quinpirole binding, had no effect on agonist-independent or quinpirole-stimulated [^{35}S]GTPγS binding. Clorgyline inhibited [^{35}S]GTPγS binding by a non-dopamine D_2 receptor-mediated mechanism. These findings demonstrate a notable discrepancy between the pharmacological profile of [^{3}H]quinpirole binding and quinpirole-stimulated [^{35}S]GTPγS binding.

Keywords: [^{35}S]GTPγS binding, agonist-stimulated; Dopamine D_2-like receptor; Quinpirole; Monoamine oxidase inhibitor; Striatum; (Rat)

1. Introduction

Quinpirole (LY 171555) is a selective agonist used in the study of dopamine D_2-like receptor-mediated effects (for review see: Clark and White, 1987). Our laboratory and others have demonstrated specific, saturable, high affinity in vitro binding of [^{3}H]quinpirole in rat striatal membranes and in autoradiographic studies, which is appropriate for the classical dopamine D_2 receptor with respect to pharmacological profile, guanyl nucleotide regulation, and regional distribution of binding sites (Seeman and Schaus, 1991; Levant et al., 1992, 1993a). Surprisingly, several compounds with low affinity for dopamine D_2-like sites, most notably monoamine oxidase inhibitors, inhibit equilibrium [^{3}H]quinpirole binding in rat striatal membranes in vitro (Levant et al., 1993b, 1996). This suggests that certain monoamine oxidase inhibitors interact with a novel binding site in rat brain, which is either labeled by [^{3}H]quinpirole in addition to dopamine receptors or which modulates [^{3}H]quinpirole binding at dopamine D_2-like receptors. This site is of particular interest as it appears to modulate behavioral sensitization to quinpirole (Culver and Szechtman, 1997; Culver et al., 2000).

Recently, dopamine receptor agonist-stimulated [^{35}S]GTPγS binding, a functional measure of G protein activation, has been described in rat striatal membranes (Geurts et al., 1999; Rinken et al., 1999). With the aim of elucidating potential mechanisms by which monoamine oxidase inhibitors might modulate the actions of quinpirole, we determined the effects of dopaminergic compounds and monoamine oxidase inhibitors on quinpirole-stimulated [^{35}S]GTPγS binding. We will show that although both classes of compounds inhibit [^{3}H]quinpirole binding in vitro, only the dopaminergic compounds modulate quinpirole-stimulated [^{35}S]GTPγS binding.

2. Materials and methods

2.1. Materials

All test compounds were purchased from Research Biochemicals (Natick, MA) with the exception of clozap-

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ine, which was a gift from Sandoz (Basel, Switzerland). GDP and GTPγS were purchased from Sigma (St. Louis, MO). [^{35}S]GTPγS (S.A. = 1250 Ci/mmol) was purchased from NEN Life Sciences Products (Boston, MA). Brains of adult, male, Sprague–Dawley rats were purchased from Harlan Industries (Indianapolis, IN).

2.2. Membrane preparation

Striata were isolated by free-hand dissection on ice, pooled and weighed. The tissue was homogenized using a PRO homogenizer in 20 volumes ice cold 2 × assay buffer (40 mM HEPES, 200 mM NaCl, 6 mM $MgCl_2$, pH 7.4). The homogenate was centrifuged at 39,800 × *g* for 10 min at 4°C, the supernatant decanted, and the pellet resuspended in 20 volumes 2 × assay buffer. After homogenization, the pellet was incubated at 37°C for 10 min to degrade endogenous dopamine. The suspension was centrifuged, resuspended, aliquoted into microfuge tubes, and re-centrifuged. Membrane pellets were stored at −70°C for subsequent use.

2.3. [^{35}S]GTPγS binding assays

A membrane pellet was thawed on ice and resuspended in 2 × assay buffer to yield a final concentration of 6 mg original wet weight per milliliter (~200 μg protein/ml). Dithiothreitol (2 mM) was added to the 2 × assay buffer immediately before use. Assays were performed in triplicate in 12 × 75 mm polystyrene tubes in a final volume of 800 μl. The optimized reaction mixture contained 300 μM GDP, ~0.1 nM [^{35}S]GTPγS, ~50 μg membrane protein, and various concentrations of test compounds. Test compounds and GDP were prepared fresh daily and diluted in dH_2O to yield a final assay buffer composition of 20 mM HEPES, 100 mM NaCl, 3 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.4. Reaction tubes were incubated at 37°C for 20 min in a shaking water bath. The reaction was terminated by rapid vacuum filtration through pre-wetted Whatman GF/B filters using a Brandel cell harvester. Filters were washed three times with 2 ml ice-cold dH_2O and placed in scintillation vials with 3-ml scintillation cocktail. Samples were allowed to equilibrate for 4 h, mixed by inversion, and counted in a Beckman Model 6500 scintillation counter. Protein concentrations were determined by the BCA method (Pierce, Rockford, IL).

Non-specific binding was defined with 10 μM unlabeled GTPγS and was subtracted from all samples. Basal [^{35}S]GTPγS binding (agonist-independent binding) was defined as the specific binding obtained in the absence of agonist. For studies of [^{35}S]GTPγS binding stimulated by a near-maximal concentration of quinpirole, the concentration of quinpirole was 30 μM.

2.4. Data analysis

[^{35}S]GTPγS binding in each sample was calculated as femtomole bound per milligram protein. Data are presented as percent stimulation over basal:

$$\%\text{Stimulation} = \left(\frac{\text{Stimulated binding} - \text{Non-specific binding}}{\text{Basal binding} - \text{Non-specific binding}} \times 100\% \right) - 100\%$$

Unless otherwise noted, data represent the mean ± S.E.M. of at least three independent determinations. The EC_{50}, IC_{50}, and E_{max} values were calculated by non-linear regression analysis using a four-parameter model (Sigma Plot, SPSS, Chicago, IL).

3. Results

The effects of dopaminergic compounds and monoamine oxidase inhibitors on quinpirole-stimulated [^{35}S]GTPγS binding were determined in rat striatal membranes. Quinpirole produced a concentration-dependent increase in [^{35}S]GTPγS binding with an E_{max} of 40 ± 0.8% over basal and an EC_{50} of 8.5 ± 0.7 μM.

The ability of four dopamine receptor antagonists to inhibit quinpirole-stimulated [^{35}S]GTPγS binding was assessed. The dopamine receptor antagonists inhibited quinpirole-stimulated [^{35}S]GTPγS binding with the following rank order of potency: spiperone > haloperidol > clozapine > SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) (Table 1).

Table 1
Pharmacological profile of quinpirole-stimulated [^{35}S]GTPγS binding in rat striatal membranes: comparison with radioligand binding
[^{35}S]GTPγS binding was determined in the presence of quinpirole (30 μM) and 11 concentrations of test compound (10^{-11} to 10^{-6} M or 10^{-9} to 10^{-4} M). Quinpirole-stimulated [^{35}S]GTPγS binding results represent the mean ± S.E.M. of five to eight independent determinations.

Compound	Quinpirole-stimulated [^{35}S]GTPγS binding IC_{50} (nM)	[^{3}H]Quinpirole binding[a] K_i (nM)
Dopamine antagonists		
Spiperone	1.3 ± 0.3	6.6 ± 0.1
Haloperidol	5.1 ± 1.2	23 ± 1
Clozapine	250 ± 58	608 ± 38
SCH 23390	> 10,000	2,367 ± 244
Monoamine oxidase inhibitors		
Clorgyline	> 10,000	21 ± 8
Ro 41-1049	> 100,000	33 ± 12
(−)-Deprenyl	> 100,000	227 ± 60
(+)-Deprenyl	> 100,000	589 ± 56

[a] Data previously reported in Levant et al. (1992, 1993b).

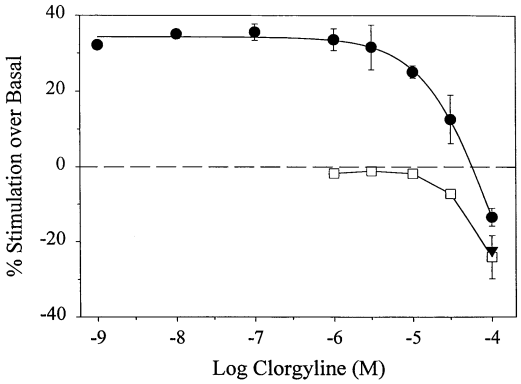

Fig. 1. Effects of clorgyline on basal and quinpirole-stimulated [^{35}S]GTPγS binding. The effects of clorgyline (10^{-9} to 10^{-4} M) were determined on agonist-independent (basal) [^{35}S]GTPγS binding and on [^{35}S]GTPγS binding stimulated by quinpirole (30 μM). The effect of clorgyline (100 μM) on basal [^{35}S]GTPγS binding was also determined in the presence of haloperidol (10 μM) ($n = 2$). All other results shown represent the mean ± S.E.M. of three independent determinations. The dashed line indicates agonist-independent (basal) [^{35}S]GTPγS binding. Quinpirole-stimulated [^{35}S]GTPγS binding (●), agonist-independent (basal) [^{35}S]GTPγS binding (□), agonist-independent [^{35}S]GTPγS binding in the presence of haloperidol (10 μM) (▼).

All antagonists failed to alter basal (agonist-independent) [^{35}S]GTPγS binding at concentrations up to 100 μM (data not shown). It was thus concluded that these compounds lacked efficacy and are ''silent'' antagonists in this model.

Because monoamine oxidase inhibitors have been shown to inhibit [^{3}H]quinpirole binding in rat striatal membranes (Levant et al., 1993b, 1996), the effects of several monoamine oxidase inhibitors on agonist-independent and quinpirole-stimulated [^{35}S]GTPγS binding were determined. Monoamine oxidase inhibitors Ro 41-1049 (*N*-(2-aminoethyl)-5-(3-fluorophenyl)-4-thiazolecarboxemide), and (+)- and (−)-deprenyl (10^{-9} to 10^{-4} M) were found to be without effect on either basal (data not shown) or quinpirole-stimulated [^{35}S]GTPγS binding (Table 1). Clorgyline inhibited both agonist-independent (basal) and quinpirole-stimulated [^{35}S]GTPγS binding in parallel at high concentrations (Fig. 1). Clorgyline-mediated inhibition of basal [^{35}S]GTPγS binding was not blocked by haloperidol (10 μM).

4. Discussion

In addition to exhibiting a classical dopamine D_2-like pharmacological profile (Levant et al., 1992, 1993a), the binding of [^{3}H]quinpirole in rat striatal membranes in vitro is inhibited by certain non-dopaminergic compounds, most notably monoamine oxidase inhibitors, by a mechanism that does not appear to involve the enzymatic activity of monoamine oxidase (Levant and Bancroft, 1998; Levant et al., 1993b, 1996). These monoamine oxidase inhibitors do not inhibit binding of other dopamine D_2-like receptor agonists or antagonists, such as [^{3}H](−)-*N*-*n*-propylnorapomorphine and [^{3}H]spiperone, suggesting that this interaction may be unique to quinpirole (Levant et al., 1993b). Although some monoamine oxidase inhibitors possess high affinity for the *sigma* and imidazoline sites, their affinities at these sites correlate poorly with their affinity in competition with [^{3}H]quinpirole (Levant et al., 1993b, 1996). These observations suggest that certain monoamine oxidase inhibitors interact with a novel binding site in rat brain, which is either labeled by [^{3}H]quinpirole in addition to dopamine receptors or which modulates [^{3}H]quinpirole binding at dopamine D_2-like receptors. Interestingly, the monoamine oxidase inhibitor clorgyline, which has high affinity in competition with [^{3}H]quinpirole (Levant et al., 1993b), blocks locomotor sensitization to quinpirole in rats (Culver and Szechtman, 1997). In contrast, moclobemide, a monoamine oxidase inhibitor with low potency in competition with [^{3}H]quinpirole (Levant et al., 1996), failed to block sensitization while producing similar neurochemical alterations to clorgyline attributable to inhibition of monoamine oxidase (Culver et al., 2000). With the aim of elucidating potential mechanisms by which monoamine oxidase inhibitors might modulate the actions of quinpirole, the effects of monoamine oxidase inhibitors on quinpirole-stimulated [^{35}S]GTPγS binding were assessed and compared with those of dopaminergic compounds.

Stimulation of [^{35}S]GTPγS binding by dopamine and dopamine D_2-like dopamine receptor agonists in rat striatal membranes has been recently described (Geurts et al., 1999; Rinken et al., 1999). The dopamine D_2-like agonist-induced [^{35}S]GTPγS binding most likely results from activation of G_i/G_o (Lachowicz and Sibley, 1997). In concordance with previous studies (Geurts et al., 1999; Rinken et al., 1999), quinpirole, a dopamine D_2-like receptor full agonist (Lahti et al., 1992), produced concentration-dependent increases in [^{35}S]GTPγS binding with micromolar potency. Dopaminergic antagonists inhibited quinpirole-stimulated [^{35}S]GTPγS binding with a rank order of potency similar to that observed in radioligand binding studies with [^{3}H]quinpirole and other dopamine D_2-like radioligands (Seeman, 1993). Accordingly, the potent dopamine D_2-like antagonists spiperone and haloperidol completely antagonized the [^{35}S]GTPγS binding induced by quinpirole at micromolar concentrations, whereas the dopamine D_1-like antagonist SCH 23390 was the least potent. Clozapine, an antagonist with moderate affinity at dopamine D_2-like sites, exhibited intermediate potency in the quinpirole-stimulated [^{35}S]GTPγS binding assay.

Whereas the effects of dopaminergic compounds on quinpirole-stimulated [^{35}S]GTPγS binding concurred with radioligand binding studies, the effects of monoamine oxidase inhibitors did not. The monoamine oxidase inhibitors Ro 41-1049 and (+)- and (−)-deprenyl, which potently inhibited [^{3}H]quinpirole binding (Levant et al., 1993b), were found to have no effect on either agonist-independent or quinpirole-stimulated [^{35}S]GTPγS binding.

Clorgyline, which possessed nanomolar affinity in competition with [^{3}H]quinpirole in radioligand binding studies (Levant et al., 1993b), inhibited quinpirole-stimulated [^{35}S]GTPγS binding only at very high concentrations. At these concentrations (> 10 μM), clorgyline also decreased agonist-independent [^{35}S]GTPγS binding suggesting that its effect could be due to inverse agonist activity at a receptor or non-specific interactions with the G protein. Because the effects of clorgyline on [^{35}S]GTPγS binding were not blocked by the dopamine D_2-like receptor antagonist haloperidol, it was concluded that those effects were not mediated by dopamine D_2-like receptors.

These data demonstrate a notable discrepancy between the pharmacological profile of [^{3}H]quinpirole binding and quinpirole-stimulated [^{35}S]GTPγS binding in rat striatal membranes. Whereas antidopaminergic compounds inhibited both [^{3}H]quinpirole binding (Levant et al., 1992) and quinpirole-stimulated [^{35}S]GTPγS binding with similar potencies, monoamine oxidase inhibitors, which potently inhibit [^{3}H]quinpirole binding (Levant et al., 1993b) failed to alter quinpirole-stimulated [^{35}S]GTPγS binding. Accordingly, the pharmacological profile of quinpirole-stimulated [^{35}S]GTPγS binding is consistent with that of the classical dopamine D_2-like receptor. As such, quinpirole represents an appropriate agonist for use in study of dopamine D_2-like receptor function in the agonist-stimulated [^{35}S]GTPγS binding assay. The mechanism underlying the discrepancy between the pharmacological profile of [^{3}H]quinpirole binding and quinpirole-stimulated [^{35}S]GTPγS binding must be determined in future experiments; however, this observation suggests that while monoamine oxidase inhibitors may modulate binding of [^{3}H]quinpirole, they do not appear to affect quinpirole-stimulated G_i/G_0 activation as assessed in this assay.

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